With compliments.
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THE PREVENTIVE MEASURES DIRECTED AGAINST BLACK-QUARTER

AND AN

INVESTIGATION INTO THE EFFICACY OF SETONING

BY

STEWART STOCKMAN

PROFESSOR OF PATHOLOGY ROYAL (DICK) VETERINARY COLLEGE
EDINBURGH

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(Reprinted from the Journal of Comparative Pathology and Therapeutics.)

R28667

THE PREVENTIVE MEASURES DIRECTED AGAINST BLACK-QUARTER, AND AN INVESTIGATION INTO THE EFFICACY OF SETONING.[1]

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STEWART STOCKMAN,

Professor of Pathology, Royal (Dick) Veterinary College, Edinburgh.

AT the present day it is known to all of us that black-quarter is a bacterial disease, and most of us, I think, believe that in the majority of cases it is contracted by inoculation of a wound in some part of the body. For the discovery of the bacillus of black-quarter, or charbon symptomatique, as it is called in France, we are indebted to Arloing, Cornevin, and Thomas. In 1880 they described and demonstrated the pathogenic properties of this bacillus, which they named the bacterium Chauvœi. It is true that in 1860 Feser described bacilli in the juice from black-quarter muscle, but Arloing, Cornevin, and Thomas were the first to give a proper account of the micro-organism. Since then they have furnished us with the greater part of our information regarding the pathology of this disease, and they have put into practice a method of preventive inoculation which has obtained great favour among continental veterinarians.

Their method of preventive inoculation, however, is not the only one in use. Among those who have done important work on this subject are Professor Kitt of Munich, and Dr Roux of Paris. These methods of prevention have not gained favour in the United Kingdom. It is only fair, however, to say that for good reasons they have not been extensively tried in this country. These reasons are—firstly, that the results of the first experiments were disastrous; secondly, that the wholesale inoculation of cattle against black-quarter is unwarrantable, because the disease is only known on certain farms, and even the warmest advocates of the present methods have to number some deaths from inoculation in their statistics. In this country one of the oldest and most popular of preventive measures directed against the disease is setoning, and some investigations which I have made into the efficacy of that measure will constitute a considerable part of this paper.

I shall discuss shortly each preventive method in use, but, before doing so, it will be as well to say a few words about the distribution of the disease, the virulent material, the susceptibility of animals, and the manner whereby they contract it, because a knowledge of these subjects is necessary before attempting to apply preventive measures.

I would like to explain here that our warfare is not accomplished when we have found even a good method of preventive inoculation.

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[1] Read before the Midland Counties Veterinary Association at Birmingham on the 27th August, 1897.

We should regard that method rather as a barrier to stem the tide, while we are applying more radical measures which take a longer time to operate.

Distribution of Black-quarter.—The disease in this country is confined to certain districts, and often in these districts to certain farms.

Veterinarians in different parts of this country will point out to you black-quarter farms with neighbouring establishments which are practically free from the disease. In some parts it would be well worth an intending tenant's while to inquire into the reputation of the farm in this respect. Strebel, in his valuable communication to the Sixth International Veterinary Congress, says that the soils in black-quarter districts have a common character. They are of a clayey nature, hence impervious and damp. This, of course, simply means that filth, including disease germs, is not drained away from such a soil, and therefore the plague is kept up for a long time. It applies to the germs of other diseases as well as that of black-quarter. Hess, after inquiring into the distribution of the disease in various parts, could not admit that any one kind of soil was particularly favourable. We know, however, that one of the best preventive measures is to drain the land, but unfortunately that is not always practicable.

It does not come within the scope of this paper to describe all the characters of the black-quarter bacillus, but I should like just to remind you here that it is a sporulating microbe, an obligatory anærobe, and that its tendencies seem to be decidedly saprophytic. On the surface of the soil the bacilli, and probably for that matter the spores as well, do not remain active for any very long time. The combined effect of oxygen and sunlight is powerfully destructive.

They sink into the ground, however, and there is evidence to show that the spores at least, if not the bacilli, may here remain active for years.

With the earth taken from the damp parts of black-quarter pastures at a depth of 12 inches, Feser succeeded in inoculating sheep with black-quarter. The earth from the dry parts did not contain the pathogenic agent. Feser's examinations seemed to show that the bacillus itself is present in the soil, but of this more evidence is required. There are two possible explanations of how these sub-soil germs are brought to the surface again. The one is that it is connected with the rise and fall of the ground water (so-called), the other is that, when the ground cracks, as it does in hot weather, the dry sub-surface earth gets blown or scraped to the top. These explanations savour somewhat of the hypothetical, but I think they are worthy of further investigation.

Animals attacked.—The animals attacked by black-quarter are chiefly cattle and sheep. The goat is also susceptible, but the horse enjoys an almost complete immunity. Of the animals commonly used for experiment in the laboratory, the guinea-pig is the best reagent. The rabbit has a certain amount of immunity, but this can be overcome by injecting along with the virus such agents as lactic or acetic acids, certain other microbes, or the toxines of the black-quarter bacillus itself.

The micrococcus prodigiosus overcomes the immunity in the rabbit, but seems to strengthen the resistance of the guinea-pig. Bruising of the tissues at the seat of inoculation seems also to intensify the action of the virus. These agencies are believed to act by taking up the attention of the white cells and the other resisting forces of the animal organism.

It is interesting to know that certain agents increase the virulence of the black-quarter microbe, or rather let us say diminish the resistance of the animal organism to it, for it helps to explain how the disease can be more virulent in one outbreak than in another. Young calves below the age of six months seem to possess a relatively high degree of immunity, but it is by no means absolute. I dare say many of us have seen the disease affect unweaned calves. The period of life at which cattle are most susceptible is from nine months to the third year. After the third year the susceptibility diminishes, but again the degree of immunity enjoyed by older animals is not absolute.

According to Messrs Arloing, Cornevin, and Thomas, the animals bred in black-quarter districts very often have a high degree of immunity, and they suggest that these animals may have acquired this as the result of one or two mild attacks of the disease.

Virulent Matter.—As regards the virulent material, the juice of the affected muscles and the œdematous fluids contain the microbe. The same applies to the fluids from the cavities. The pulps of internal organs are virulent, and so are the bloody discharges from the nostrils and anus. It is chiefly by the latter that the pastures are reinfected. The blood contains few microbes. That it does contain a few, however, has been shown by Nocard.

As the subject of this paper is prevention, I would like here to call your attention to one measure that is entirely neglected, viz., disinfection of the spot where the animal is found dead. One cannot find out every part where the patient has been, but the places where they have lain down to die will be the most contaminated. It has been my habit for several years to advise disinfection of these spots, but I have never received any communications bearing on its efficacy. The best agents for this purpose are corrosive sublimate, 1–2000 in water; or carbolic acid, 5 per cent. aqueous solution.

Infection.—It has been experimentally demonstrated that the black-quarter virus (dried muscle juice introduced under the skin or by the alimentary tract), when a sufficient quantity is used, gives the disease to susceptible animals.

Inoculation into the muscular tissue is still more certain.

Injection into the trachea or veins excites only a rise of temperature and slight indisposition. After recovery the animal is endowed with the highest degree of immunity. Unfortunately this method cannot be applied in practice, because, should a drop of the bacterium or spore containing fluid get into the subcutaneous tissues, a fatal termination from black-quarter may be the result. Further, if while the germs of the disease are circulating in the blood a bruise be inflicted on the animal, so that some blood escapes into the tissues, the animal may die of black-quarter, just as if it had been inoculated subcutaneously from without (Arloing, Cornevin, and Thomas).

The blood, however, is a bad medium for the anærobic bacilli, and they do not flourish in it. Still, during a natural attack of the disease they seem to invade the blood-stream in small numbers, too few to be demonstrated in cover-glass preparations, though the blood can yet serve for the inoculation of culture media. As further evidence of their existence in the blood-steam, we have the occurrence of tumours away from the seat of inoculation and the presence of bacilli in the cavities. On *a priori* grounds, then, natural infection seems to operate by ingestion of spore-contaminated material, or by the spore gaining access to the subcutaneous tissues through a wound. I incline to think that the latter method is the more common, because the disease is relatively rare in coated animals like the sheep, although it is the most susceptible of all to inoculation.

Infection does not operate equally well in every part of the body.

We seldom get evidence of the virus having penetrated by those parts where the connective tissue is dense, and it has been shown experimentally that it is more difficult to obtain positive results by inoculating at parts such as the tail or the ear.

In the practice of preventive inoculation advantage has been taken of this observation. Fewer animals die from the inoculation when performed at the extremity of the tail. However, this method has its disadvantages. Accidents similar to those which followed preventive inoculation against pleuro-pneumonia are said to be fairly frequent. Moreover, it is not easy to insert a needle at this part, both on account of the movements of the animal and the density of the tissues. The other parts where the operation is practised are behind or in front of the shoulder.

Methods of Preparing the Vaccine (Arloing, Cornevin and Thomas).

The black-quarter virus in the dried state is very resistant to the action of heat. A dry temperature of 110° C. continued for six hours is necessary to completely destroy it. The same temperature when got by superheated steam destroys it in twenty minutes. Arloing, Cornevin, and Thomas say that the dry virus must be exposed to a dry temperature of 85° C. for six hours to obtain a solid diminution of virulence.

Between these ranges of temperature one can obtain virus of varying strength. To obtain their vaccine they proceed as follows. A portion of the affected muscle is bruised in a mortar with a few drops of water. The liquid containing the virus is filtered through muslin. It is then spread in a thin layer on a plate and dried at 37° C. After drying, one scrapes the plate and obtains reddish-brown scales or powder. This virus remains active for a long time. To attenuate its virulence one adds two parts of water to one of powder. The mixture is spread out on a plate in a thin layer, and exposed to a dry temperature of from 100-104° C. for seven hours. A brownish scaly material is afterwards scraped off the plate, and this is the first vaccine.

A second and stronger vaccine is obtained by exposing the virus similarly prepared to a temperature of from 90-95° C. for seven hours. The only agent that can possibly resist this temperature is the spore, but it becomes attenuated. The reddish material is powdered finely and sent out in packets. Before use, one puts the powder in a sterilised mortar (boiled fifteen minutes), and adds sterilised water (boiled) to the amount of 10 cc. The mixture is then filtered through muslin which has been previously boiled.

Arloing, Cornevin, and Thomas advise the inoculation to be performed at about 3 inches from the extremity of the tail. The dose for oxen older than eighteen months is 18 mm., and from 10-15 mm., according to size, for younger animals. The second vaccine is inoculated ten days after the first, and, of course, the operation is conducted as far as possible with antiseptic precautions.

Strebel has for some years inoculated this vaccine in the neighbourhood of the shoulder with good results, and lately it has been advised to perform the operation on the side of the chest.

Professor Kitt of Munich prepared a vaccine by exposing the virulent brown powder to a steam temperature of from 98-100° C. for about six hours. He found that sheep and oxen withstood a dose of from ·02-·5 gramme, and that afterwards they presented a higher degree of immunity. The strength of this vaccine is midway between the first and second vaccines of Arloing, Cornevin, and Thomas. Kitt's object was to endow the animals with immunity by a single inoculation, and thus reduce the trouble and expense connected with the operations. In practice, however, Kitt's method has not realised his expectations. Kitt himself has said that his own method is less surely protective than that of Arloing, Cornevin, and Thomas. Recently Kitt recommended the use of a vaccine prepared from pure cultures of the black-quarter bacillus, but the results have been by no means encouraging.

Valuable statistics of the results of preventive inoculation were presented at the Sixth International Veterinary Congress, 1895, by Prof. Cornevin of Lyons, Prof. Hess of Berne, and M. Strebel of Fribourg. I shall confine myself to the most important of them.

Cornevin records the results of 21,854 inoculations during one-and-a-half years 1893-95, with a death-rate from the operation (*i.e.*, due to inoculated black-quarter)(= ·0823 per cent). He says, however, that the death-rate varied considerably in different districts. Still, in every case the operation appears to have reduced the death-rate below what it was before the introduction of preventive inoculation. He gives the mortality from black-quarter in nineteen districts before and after inoculation was introduced. That before varied from 25 per cent. to 2 per cent. Afterwards it varied from 1 per cent. to 0 per cent.

Hess records 148,569 inoculations from 1885-94. The total number of failures (*i.e.*, deaths from inoculation, or black-quarter contracted in spite of inoculation) amounts to 736 or ·5 per cent. These 736 failures are made up by numbers varying from 50 out of 13,794 in the year 1888 (= ·36 per cent.), to 84 out of 12,061 (= ·7 per cent.) in 1889.

The number of animals dead from black-quarter inoculation by the vaccine varied from 8 out of 15,137 (= ·05 per cent.) in 1885, to 36 out of 17,791 (= ·2 per cent.) in 1894. The number of deaths from black-quarter contracted naturally in spite of the inoculation varied from 31 out of 13,744 (= ·22 per cent,) in 1888, to 61 out of 12,061 (= ·51 per cent.) in 1889. One sees, then, that the number of failures is very variable, and when the bulk of them have to be recorded in one place, as sometimes happens, one's faith in the method is apt to be shaken. At least, one is diffident about advising a client to adopt it.

According to Hess the number of deaths following the first inoculation is almost two-thirds of the total number that die from inoculation black-quarter. A weaker first vaccine is then indicated, but this if prepared in the same way would probably raise the death-rate after the second inoculation, or render a third operation necessary. Lastly, Hess gives a table comparing the failures following the operations at the tail with those at the shoulder.

In 1894, 17,791 animals were inoculated, viz., 12,299 (=69 per cent.) at the tail, and 5,442 (= 31 per cent.) at the shoulder.

Inoculation at the tail gave 42 failures, viz., 17 deaths from inoculation black-quarter, and 25 from the disease naturally contracted. This gives a total of ·32 per cent., made up of ·13 per cent. from inoculation, and ·19 per cent. from natural black-quarter.

At the shoulder there were 51 failures (= ·93 per cent.), viz., 19 deaths from inoculation (= ·35 per cent.), and 32 deaths from natural black-quarter (= ·58 per cent.).

The reports by Strebel are the fullest and most interesting of all.

In Table II. he gives the results of 192,948 inoculations performed in nineteen cantons or countries from 1884-94, and he compares the death-rate from black-quarter in the vaccinated with that in 315,168 non-vaccinated animals which frequented the same pastures.

The total number of failures, from inoculation and from the disease contracted in spite of it, amounted to 922 or ·48 per cent. The highest death-rate was 30 out of 2255 animals vaccinated (= 1·33 per cent. in Uri), and the lowest was 0 out of 212 in Soleure.

Inoculation was responsible for 82 deaths (= ·042 per cent.). The highest rate was 5 out of 680 animals (= ·737 per cent.) in Haute-Autriche. The lowest rate was 0 out of 6905 in Glaris. The disease contracted in spite of inoculation was responsible for 840 of the deaths, the largest proportion being 30 out of 2255 (= 1·33 per cent.) in Uri, and the lowest 0 out of 212 in Soleure. Out of 315,168 non-vaccinated animals going on the same pastures, 5482, or 1·74 per cent., died of black-quarter. The highest rate was 13 out of 184 (= 7·06 per cent.) in Soleure, and the lowest was 89 out of 13,756 (= ·65 per cent.) in Carinthia.

In Table IV. he gives the result of 129,740 inoculations performed at the extremity of the tail, and compares the death-rate with that in 234,560 non-vaccinated animals on the same pastures.

The total number of failures was 585 (= ·46 per cent.). The highest rate was 1·33 per cent. in Uri, and the lowest 0 per cent. in Soleure.

These cases are also included in Table II. From inoculation there were 35 deaths (= ·027 per cent). The highest rate was 6 out of 3951 (= ·152 per cent.) in Vaud, and the lowest was 0 out of 6909 in Glaris. From black-quarter naturally contracted there 550 deaths (= ·42 per cent.).

The highest rate was 30 out of 2255 (= 1·33 per cent.) in Uri, and the lowest was 0 out of 212 in Soleure. Of the non-vaccinated 4136, or 1·76 per cent., died of the disease. The highest rate was 13 out of 184, or 7 per cent., in Soleure; the lowest was 89 out of 13,756, or ·65 per cent., in Carinthia.

In Table VI. the results of 62,203 inoculations performed at the shoulder are given, and the death-rate is compared with that in 82,334 non-vaccinated animals on the same pastures. The number of failures amounted to 327, or ·52 per cent.; the highest rate was 106 out of 8865, or 1·19 per cent., in Styria; and the lowest was 9 out of 6732, or ·13 per cent., in Salzbourg. The inoculation accounted for

45 deaths (= ·072 per cent.); the highest rate was 5 out of 680, or ·735 per cent. in Haute-Autriche, and the lowest 0 out of 5758 in Hungary. The disease naturally contracted killed 282, or ·45 per cent., of the vaccinated; the highest rate was 91 out of 8865, or 1·02 per cent., in Styria; the lowest 6 out of 6732, or ·09 per cent., in Salzbourg. Of the non-vaccinated 1350, or 1·64 per cent., died of black-quarter; the highest rate was 172 out of 6254, or 2·76 per cent., in Haute-Autriche; and the lowest 169 out of 17,854, or ·95 per cent., in Styria.

In Table VII. are given the results of the single inoculation method performed at the shoulder in 53,656 cases.

The number of failures was 258, or ·48 per cent. Inoculation killed 67, or ·125 per cent.; the highest rate was 12 out of 1429, or ·840 per cent., in Basse-Autriche, and the lowest 0 out of 962, in Fribourg. In spite of the vaccination 191, or ·350 per cent. of the animals, died of the disease. The highest rate was 91 out of 8865, or 1·03 per cent., in Styria; the lowest was 0 out of 12,300 in Algeria.

In Table IX. we find the results of 34,756 inoculations performed at the shoulder with the dry vaccine of Kitt (*i.e.* a single inoculation with a virus midway in strength between the first and second vaccines of Arloing, Cornevin, and Thomas). The death-rate is compared with that in 65,518 non-vaccinated animals on the same pastures. The total loss from black-quarter in the vaccinated amounted to 203, or ·58 per cent.; the highest rate being 5 out of 392, or 1·27 per cent., in Haute-Autriche; the lowest 9 out of 6732, or ·13 per cent., in Salzbourg.

The operation was responsible for 42 deaths, or ·121 per cent. The highest rate was 1·275 per cent. in Haute-Autriche; the lowest ·044 per cent. in Salzburg.

Naturally contracted black-quarter accounted for 161 deaths, or ·46 per cent. The highest rate was 1·02 per cent. in Styria; the lowest 0 out of 392 in Haute-Autriche. Of the non-vaccinated 992, or 1·51 per cent., died of the disease. The highest rate was 1·91 per cent. in Haute-Autriche; the lowest ·95 per cent. in Styria.

In Table X. we find the results of 5644 inoculations performed with the pure culture vaccine of Kitt (the virus is injected once at the shoulder).

The death-rate is compared with that in 13,183 non-vaccinated animals on the same pastures.

In the vaccinated the total loss was 89, or 1·58 per cent. The inoculation killed 8, or 1·41 per cent.; the disease naturally contracted killed 81, or 1·43 per cent.

In the non-vaccinated, 191, or 1·45 per cent., died of black-quarter.

Lastly, Strebel gives a Table (XI.) in which are compared the results obtained from the different methods.

[Table.

Method.	*No. of Animals Vaccinated.*	*Died.*					
		From Inoculation.	*Per Cent.*	*From naturally Contracted Black-Quarter.*	*Per Cent.*	*Total.*	*Per Cent.*
Double inoculation at tail	325,893	188	0·056	1245	0·38	1430	0·44
Total inoculations in region of shoulder	91,066	76	0·084	365	0·40	441	0·4
Double inoculation in region of shoulder	37,410	8	0·022	157	0·42	165	0·44
Inoculation (single) behind shoulder with the dry vaccine of Kitt	39,084	61	0·156	187	0·48	248	0·63
Single inoculation with the pure culture vaccine of Kitt	5,643	8	0·141	81	1·43	89	1·58

Some apparent relation can be traced between the severity of the inoculation and the number of animals which contracted the disease naturally afterwards. As a rule, where the death-rate from inoculation is high, the number of failures from insufficient protection is relatively small.

These percentages are very valuable, but I do not think that the laws of proportion can be given their full arithmetical significance in a case of this kind, because there are several factors, such as variation in virulence and susceptibility, about which we have too little information. We know that in certain parts the operations have been followed by many fatal results, that on this account the practice of preventive inoculation against black-quarter was abandoned in England, and that the degree of immunity derived from it has been in many cases insufficient to ward off a spontaneous attack of the disease. The latter part of the resolution voted by the Sixth International Veterinary Congress appears to me to be an excellent criticism on the applicability of the method ; it is as follows :

"The Veterinary Congress regards preventive inoculation against black-quarter, discovered by Messrs Arloing, Cornevin, and Thomas, as a precious means of combating the disease, and practicable in the countries where compensation provides for the losses following inoculation."

Setoning as a Preventive of Black-Quarter.

One of the oldest measures directed against the disease in this country is setoning. The operation is known to all ; I need say nothing about that part of it. I have been unable to discover who was the originator of the idea,[1] nor have I met with any statistical report on its efficacy. However, it is widely practised at the present day in black-quarter districts. Many veterinarians of long experience put great faith in it as a preventive, others practise it on the grounds that they must do something, and setoning is the only means they have at hand. I think I may say that the majority of practitioners put some faith in it, at least such an opinion I have come to after many inquiries.

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[1] The seton method is mentioned by John Lawrence in "A General Treatise on Cattle," 1809. Francis Clater "Every Man His Own Cattle Doctor," 1825. Steel "On the Ox," 1881.

They say that experience has taught them that the disease frequently stops after a herd has been setoned. They have no explanation to offer regarding its action; they simply fall back on the r experience.

This method of reasoning may well be called in question, especially when dealing with a disease like the one under discussion, for everyone knows that a single animal in a herd may die of black-quarter and its companions remain healthy whether they are setoned or not. Evidently the disease is not very contagious in the ordinary sense of the word. It does not pass from animal to animal as pleuro-pneumonia seems to do; it is to be classed rather with such bacterial diseases as tetanus. There are three tests which might have been applied to the method. Firstly, that of comparing the death-rate in a black-quarter district for a number of years before setoning was introduced, with that during several years after its adoption. This, however, would have given misleading results in many districts, because the disease has almost entirely disappeared from certain farms on account of improved treatment of the land. Secondly, a number of setoned animals might have been pastured with the unsetoned on black-quarter farms, and the death-rate from black-quarter compared in the two sets of animals. To make such a test of any value a great number of animals would have to be kept under observation over several years, and there would have to be a decided difference in the mortality of the two sets of animals before an opinion could be formed one way or the other. The third test is one which according to our present notions should be the initial one. It is to inoculate with equal doses of the same virulent material one lot of susceptible animals that have been setoned, and a second lot not setoned but otherwise kept under the same conditions as the first, in order to see whether the setoned lot withstand the inoculation entirely or for a longer period than the unsetoned. A combination of the second and third tests would be the most satisfactory of all. My investigations have dealt entirely with the experimental test.

It appeared to me that one could not disregard entirely the evidence of experience, such as it is. I did not think that the mere setoning could possess of itself any virtues, but with others I thought it possible that the products of the microbes of the suppuration which follows the operation might have the property of endowing the animal organism with some degree of immunity. This idea is not purely imaginary. Roux has shown that animals rendered immune against black-quarter itself are also immunised against septicæmia. The Midland Veterinary Association in 1891 put the setoning method to an experimental test, while they were conducting other experiments on the efficacy of protective inoculation with the Lyon's and the Kitt vaccins. When I began my experiments I did not know that the efficacy of the setoning method had been tested in any way, but I purposely selected my animals from a very susceptible species, viz., the sheep, and was thus spared the disappointment of seeing the control animals survive the others except in one case. In the experiments undertaken by the Midland Veterinary Association the setoning method was tested on calves, animals whose susceptibility is very variable. Indeed, it cannot be denied that the majority of calves possess a high degree of immunity from black-quarter. The result of the above-mentioned experiments was to leave the question practically in *statu quo*. My first experiments were undertaken to see if the products of pyogenic microbes had any protective action. I obtained two yearling sheep (ewes). One was kept as a control, the other received hypodermically on the 24th November 1896, at 1.30, 6 cc. of an eleven-days'-old broth culture of the staphylococcus pyogenes aureus from which the microbes had been removed by passing it through a porcelain filter. The normal temperature of this ewe was 104° F. There was no rise of temperature after injection. It is needless to say that the surgical operations in all cases were performed with strict antiseptic precautions.

I thought it might be of interest to observe if the dissolved products of the staphylococcus aureus had any effect on the number of white cells in the blood, for everyone knows the important part assigned to the phagocyte in the production of immunity. The colourless corpuscles in the blood of this animal numbered 18,400 per cub. mm. before the experiment.

26th November, 11 A.M. 10 cc. of same toxin (eleven days' culture) were injected hypodermically. 12 P.M., temperature 104·6°. White cells 16,800 per cub. m.

27th November, 1.30 P.M. 12 cc. of same toxin injected. White cells 16,800 per cub. m.

28th November. Temperature, 104° F.

29th November, 11 A.M. 16 cc. of same toxin injected directly into jugular vein.

12 P.M. Temperature, 105° F. White cells 17,600 per cub. m.

1st December, 12 P.M. 20 cc. of same toxin into jugular vein.

12.30 P.M. White cells 16,000 per cub. m. (vein).

6th December. Temperature, 104° F. 30 cc. of filtered broth culture of staphylococcus pyogenes aureus, after fourteen days' incubation, were injected into jugular vein, and 4 cc. hypodermically. (This culture was made from the pus of an experimentally produced abscess.) White cells twenty minutes after the injection numbered 9600.

I do not wish to lay too much stress on these observations on the white cells, since I have not made a sufficient number of observations. So far, however, they seem to show that injection of large quantities (30 cc.) of pus toxin into the veins, is followed by a temporary diminution in the number of white cells of the blood. From the 24th November to the 6th December this ewe received 98 cc. of pus toxin, 32 cc. under the skin, and 66 cc into the veins.

10th December. 5 cc. of sterile bouillon were mixed with the subdermic fluid of a guinea-pig dead of black-quarter, and 1 cc. of this mixture was injected into the shoulder of the ewe.

11th December, 10.30. Ewe very ill, cannot stand, temperature 105° F., comatose. The animal died of black-quarter some time in the evening of the 11th.

On the 10th December a control yearling ewe, which had been kept all the time under the same conditions as the other, except that it received no pus toxin, was inoculated with 1 cc. of the same mixture of bouillon and black-quarter virus. The temperature of this animal rose from 104° F. at the time of the injection to 105° F. on the next day. There was no perceptible tumour at the seat of inoculation, and the animal remained alive and well.

It may be objected that the control ewe had a greater natural resistance than the other. If it had, the experiment at least shows that this resistance was greater than that conferred on its fellow by the pus toxin, which was nil.

I am inclined to believe, however, that the pus toxin lessened the natural resistance of the other animal. I may say, that I tried afterwards to overcome the now reinforced immunity of the control by injecting pus toxin, but failed. I have also performed three similar experiments on guinea-pigs, using the staphylococcus pyogenes aureus itself as well as its products obtained by filtration. In no case have the pus microbes or their products prolonged the life of the animals injected with black-quarter virus.

I thought, however, that it would be more satisfactory if experiments were performed on animals that had been actually setoned, since their condition would then correspond to that of animals presumably immunised by setoning, for it appeared quite possible that the animals might derive some degree of immunity from microbes other than the staphylococcus pyogenes aureus.

Accordingly, I obtained three healthy yearling ewes. One of them (A) was setoned in the neck, according to the usual method, without any antiseptic precautions. Another (B) was setoned according to the rules of antiseptic surgery, and the wound was kept aseptic by covering with a pad of corrosive sublimate wool, which was soaked every day with creoline wash. At no time during the experiment was there any sign of suppuration in this animal. The third ewe (C) was kept as a control. The animals were lodged in a roomy loose-box, and fed on oats, maize, bran, hay, and grass.

The setons were allowed to act for a month. The white cells of the blood were counted before and at different stages of the experiment, but they underwent no appreciable change.

On the 6th July each of the three animals received into the left thigh 1·7 cc. of an emulsion made by triturating in a sterilised mortar ·5 gr. of dried black-quarter muscle with 8 cc. of sterile water, and adding one drop of acetic acid after straining through muslin. The muscle was about one year old. The details of these experiments are as follows:—

Ewe (A) (suppuration.) 6th July, 12.30 P.M. Temperature, 103°. Injected 1·7 cc. of above emulsion into muscles of left thigh.

7th July, 11.0 A.M. Temperature, 105·6°. Leg swollen and livid at and below seat of inoculation, but not much emphysema. Animal very dull and partially comatose, could not stand, apparently dying.

Died of black-quarter at 11.30 A.M.

Ewe (B) (aseptic seton.) The object of this experiment was, of course, to see whether microbes were responsible or not for any degree of immunity that might be produced.

6th July, 12.30 P.M. Temperature, 104° F. Injected 1·7 cc. of same virus (as above) into muscles of left thigh.

7th July, 11.0 A.M. Temperature, 106°. Leg swollen and livid at and below seat of inoculation. Very lame but walking about, slight emphysema of thigh, breathing hurried.

9.0 P.M. Dull but able to walk about, does not look like dying.

11.30 P.M. Able to walk, no signs of coma.

8th July, 10.30 A.M. Temperature, 106°. Still no sign of coma, able to walk.

Died of black-quarter at 1.30 P.M.

Ewe (C) (control). 6th July, 12.30. Temperature, 104·5° F. Received 1·7 cc of same emulsion (virus) into muscles of left thigh.

7th July, 11.0 A.M. Temperature, 105° F. Leg swollen and livid at and below seat of inoculation. Lame but not so bad as others, still runs off when approached, slight emphysema at seat of inoculation.

9.0 P.M. Very ill, partially comatose. Died of black-quarter at 11.0 P.M.

It will be seen that (A) (septic seton) died in the short space of twenty-four hours, and predeceased (C) (control) by twelve hours, and (B) (aseptic seton) by twenty-four hours, while (C) died about eleven hours before (B).

The relatively early death of (A) is in accordance with the result of the experiments performed with pus toxin. The results are sufficient to convince me that setoning does not endow animals with any degree of immunity from black-quarter; in fact, there is some evidence to show that, as performed in practice, it has the opposite effect. I feel sure that if further experiments be undertaken on the same lines, the evidence against the efficacy of setoning will only become the stronger.[1] Were it possible to determine exactly what is the fatal dose of the black-quarter virus, one might experiment with greater exactitude On account of the varying degrees of individual susceptibility and for other reasons this cannot be done. The virus which I employed, however, cannot have been of excessive strength, because at the same time I inoculated ·5 cc. of the same virus into the thighs of two guinea-pigs, one protected by filtered muscle juice, the other not, without a fatal termination in either case.

I have also performed some experiments to establish the degree of immunity conferred on a susceptible animal by the filtered muscle juices from animals dead of black-quarter. When I began the latter experiments I was not aware that Roux in 1888 (*Annales de l'Institut Pasteur*) had already shown that immunity could be thus conferred. Fortunately I came across this paper before proceeding very far. Roux showed that if the affected muscles of guinea-pigs dead of black-quarter be rubbed up with half their weight of water, the liquid obtained after pressing and filtering through porcelain could confer immunity on other guinea-pigs, if injected in large doses. According to the same authority cultures of the bacillus sterilised by heating to 115° C. had the same effect, but the immunity derived from the juices was superior. This subject was again taken up by Duenchmann (*Annales de l'Institute Pasteur*) in 1894. Experimenting with guinea-pigs, he was able to confirm Roux's finding with regard to the filtered juices, but could not do so regarding the filtered cultures. His results with the latter substances led him to conclude that the toxines from cultures were the reverse of protective. This is quite in accordance with .the view that the products of the bacilli, acting like lactic acid, etc., help to overcome the resistance of an animal to black-quarter. I was proceeding in a similar, though slightly different manner. By means of a meat press I obtained the juices from the affected muscles of sheep. These juices were passed through a Chamberland filter, and then tried on guinea-pigs and sheep. The results of my experiments have been to confirm what Roux had already found in the guinea-pig, viz., that the muscle juices of animals dead from black-quarter are protective for other animals after filtration.

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[1] Since the reading of this paper I have received a very valuable observation from my friend Mr F. of B., who has taken much interest in this investigation. At the request of a client, whose losses from black-quarter are annually very high, my friend setoned fifteen yearling beasts. For some reason a sixteenth beast was not setoned. The sixteen animals were all pastured on the same meadows. All the setoned animals died of black-quarter, and were survived by the one that had not been setoned.

My object in undertaking the latter experiments was to see if it were possible to obtain a substance practically harmless to animals, but capable of enabling them to withstand such a dose of strong virus as would endow them with a higher degree of immunity than that acquired from the methods of preventive inoculation now practised. I have already pointed out that failures occur either because the vaccine is too strong or too weak. My reasons for using the muscle juice were, that it appeared to me to be the best way of getting an active toxin, and that one would get the benefit of any antitoxin substances that might be formed in the animal organism. If it were possible to get in the muscle juice such a protective substance as I have spoken of, one would have no hesitation in availing oneself of preventive inoculation even in districts where the death-rate from black-quarter is relatively very small. The amount of experimental evidence which I possess on this point is not sufficient as yet to justify a communication. So far, however, the results have been encouraging, and I hope at a future time to have the pleasure of communicating the outcome of some further experiments.

W. AND A. K. JOHNSTON, EDINBURGH AND LONDON.